IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:
Hiroyuki FUJITA
Serial Number: 09/663,709
Filed: September 18, 2000
For: ANGIOTENSIN CONVERTING ENZYME INHIBITOR

Group Art Unit: 1654
Examiner: Anish Gupta

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner
Washington, D.C. 20231

Sir,

Hiroyuki FUJITA residing at 13-1, Muroyama 2-chome, Ibaraki-shi, Osaka, JAPAN, declares and states:

1. That he graduated from Department of Applied Biological Science, Faculty of Applied Biological Science, Hiroshima University, Hiroshima, Japan, in the year 1987;

2. That he has been employed in the capacity since 1990 by Nippon Synthetic Chemical Industry Co., Ltd., Central Research Laboratory;

3. That he has been engaged in research and development on Food Science.

4. That he received the degree of Doctor of Agriculture from Kyoto University, Kyoto, Japan in the year 1996;

5. That he is the present inventor, and has read and is familiar with the instant application for United States Letters Patent and the Office Action thereto mailed August 26, 2003.

6. That he conducted the experiments described below in order to demonstrate that more than 10 % by weight of polypeptides having a molecular weight of at least 5,000 is included in Supernatant A described in Yokoyama et al., which is obtained by digesting with thermolysin and that Supernatant A has bitter taste and leaves bad aftertaste.

(Materials and Methods)

Sample

The supernatant A obtained by the method described in Biosci. Biotech.Biochem., 56 (10) ("Digestion of dried bonito" on page 1541, left column, line 29 to right column, line 9) was used as the sample for the experiment.

Five grams of dried bonito muscle was suspended in 45 ml of distilled water and homogenized with a Polytron (Kinematica GmbH PT t0/35, Switzerland) for 1 min. The homogenate was boiled for 10 min. The boiled homogenate was digested by thermolysin. Enzymatic digestion was done in the presence of 880 μg/ml of proteases at 37°C for 3 hr. Digestions were done at pH 7.5. The digests were boiled for 10 min to inactivate the enzymes. The supernatants were recovered after centrifugation (supernatant A). This supernatant A was used as the sample.

Gel filtration chromatography

Molecular weight distribution of sample was measured by a gel filtration chromatography. Concretely, using a peptide and a protein [Asp-Gly-Leu-Tyr-Pro (molecular weight 563), neurotensin (molecular weight 1637), ribonuclease (molecular weight 13700)] as s standard substance, which have the known molecular weights, a sample was eluted in the following conditions to determine a retention time, and a molecular weight was determined by using a calibration curve obtained by plotting the molecular weights on the ordinate and the retention times on the abscissa logarithmically. An amount "% by weight" of the components having a molecular weight of at least 5000 is

represented as "% area" of the peaks corresponding to components having a molecular weight of at least 5000 after dividing the peaks at the position of the molecular weight 5000 on an elution chromatogram.

(Fraction conditions)

Column : Protein Pak60 (made by Waters) 5 × 250 mm

Mobile phase : 50 % by volume acetonitrile aqueous solution containing 0.1 % by volume TFA (trifluoroacetic acid)

Flow rate : 0.7 mL/min

Detection : RI

Sample amount: 1 mg

(Relation between molecular weight of standard substance and elution time)

Standard substance	Molecular weight	Elution time (minute)
Asp-Gly-Leu-Tyr-Pro	563	31
Neurotensin	1637	26
Ribonuclease	13700	19

In the above-mentioned conditions, since a substance having a molecular weight of 5000 was eluted at 29 minutes, any substance eluted at not more than 29 minutes was regarded as polypeptide components having a molecular weight of at least 5000.

Bitterness

The sample was evaluated in terms of bitterness and taste of 1% solution as follows.

(Bitterness)

◎ not bitter

○ a little bitter
△ bitter
× too biter
(Aftertaste)
◎ leave no aftertaste but pleasant taste
○ leave little aftertaste
△ leave slightly bad aftertaste
× leave bad aftertaste

Result and Discussion

The results regarding content of polypeptides having a molecular weight of at least 5,000 in the sample and bitterness and aftertaste of the sample are shown in Table 1.

Table 1

Content of peptides having at least 5000 of molecular weight (% by weight)	21
Bitterness	△
Aftertaste	×

As shown in Table 1, supernatant A described in Yokoyama et al., which is obtained by digesting fish meat with thermolysin, was found to contain more than 10 % by weight of polypeptides having a molecular weight of at least 5000. Furthermore, when bitterness and aftertaste of supernatant A were evaluated using the same evaluation criteria as in Examples of the present invention, supernatant A was found to have bitter taste and leave bad aftertaste.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

This 11th day of December, 2003

by Hiroyuki Fujita

Hiroyuki Fujita

We, the undersigned witnesses, hereby acknowledge that Hiroyuki Fujita is personally known to us and did execute the foregoing Declaration in our presence on:

Date: December 11, 2003 Witness Yoshinori Ohata

Date: December 11, 2003 Witness Shinji Hashiwagi